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Beyond Clinical Trials in B-Cell Malignancies: What Real-World Experience Tells Us

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CAR-T: From Bed to Bench and Back Again

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Beyond Clinical Trials in B-Cell Malignancies: What Real-World Experience Tells Us

This symposium took place on the 13th June 2019, as part of the European Hematology Association (EHA) Congress in Amsterdam, the Netherlands

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Meeting Summary

The Kite, a Gilead company, symposium “Beyond Clinical Trials in B-Cell Malignancies: What Real-World Experience Tells Us” took place as part of the 24th European Hematology Association (EHA) Congress and focussed on using real-world evidence (RWE) to complement clinical trial data for B-cell malignancies. Prof New began the symposium by discussing the importance of real-world data, as well as ways in which the data can be collected and used. Prof New continued with the example of the Salford Lung Study, a pragmatic Phase III real-world effectiveness trial that collected large quantities of data from hospital and primary care electronic medical records. This allowed for considerably more data to be collected compared with regular clinical trials. These data were used to provide quick responses to inquiries by regulatory authorities. The symposium was continued by Dr Hoechstetter and Prof Ysebaert, who presented idelalisib clinical outcomes and safety results obtained from both clinical trials and real-world studies. In general, real-world studies of Zydelig (idelalisib) showed similar results to data obtained from clinical trials. Prof Lin presented clinical trial results and real-world data for treatment with YESCARTA▼ (axicabtagene ciloleucel) in patients with relapsed/refractory diffuse large B-cell lymphoma (DLBCL) and concluded that data from observational studies support clinical trial data and showed effectiveness and acceptable safety in patient populations who were excluded from clinical trials. Mr McGrath continued the symposium by presenting the European Society for Bone Marrow Transplantation (EBMT) and its involvement in patient registries for haematological malignancies. Mr McGrath showed how EBMT registries formed an integral part of recent approvals of haematologic therapies and how their registries are involved in long-term collection of safety data mandated by regulatory agencies. Finally, Prof New presented the current treatment pathway for new drug development and how this pathway can be improved by using adaptive approaches. Prof New also showed the involvement of adaptive approaches in the recent approvals of chimeric antigen receptor (CAR) T-cell therapies and how these methods led to quicker access of these therapies for patients. He explained that using data already collected during routine clinical care could supplement the data collected by EBMT to provide enhanced safety and effectiveness data for patients, clinicians, and payers.

Defining Real-World Experience

Professor John P. New

Clinical efficacy data are insufficient in today's treatment landscape, and the multitude of different stakeholders requires supplementary evidence, including a favourable risk-benefit profile, value for the money spent on treatment, as well as effectiveness in larger real-world populations once clinical trials are completed. All these requirements make the development pathway for different regulatory medicines more difficult.

Both the USA¹ and European Union (EU)² regulatory authorities now accept the need for real-world data that can then be processed into RWE. Drugs are tested in small numbers of select patients in clinical trials before receiving marketing authorisation. Upon marketing authorisation, new drugs are used in large numbers of patients, and information can be

captured using large datasets with real-world data. These data can be obtained from several sources that include routinely collected hospital data, such as case report forms; admissions data and billing information; data from claims/prescribing databases and disease registry data; and data collected from wearable technologies.^{1,2}

Adherence to medications is very different in randomised clinical trials compared with the real world, with a substantial proportion of patients with cancer being nonadherent to their oncology medications. For example, 26.4% of patients with chronic myeloid leukaemia who were receiving long-term imatinib treatment were not adherent at a median 5 years after diagnosis,³ with similar proportions of nonadherence seen in patients receiving imatinib for gastrointestinal tumours, which is in stark contrast to physician-perceived adherence rates of up to 97.0%.⁴

The Salford Lung Study was a randomised, pragmatic, Phase III, real-world effectiveness trial in patients with chronic obstructive pulmonary

disease. A total of 4,232 patients were randomised to either fluticasone furoate/vilanterol once daily or the standard therapy.^{5,6} One of the main inclusion criteria was patient consent for the investigators to use all their clinical records, including general practitioner data for the previous 3 years. Compared with randomised controlled trials for which the data footprint spans from the first subject first visit to the last subject last visit, the Salford Lung Study could collect all data from general practitioners and hospitals involved in the study. This was particularly useful for the sponsor when regulatory agents asked for supplementary data regarding events of pneumonia for regulatory approval in asthma. Although this information was not part of a prespecified endpoint, data were available and could be analysed and provided to the regulators within 3 months of the request.

RWE has been pivotal in recent regulatory submissions and is particularly important in rare diseases for which the number of patients is insufficient or where patients have an extremely poor prognosis. Recent drugs in the haematology field for which RWE data have been extremely important in initial regulatory decisions, post-marketing extension of indications, and post-marketing commitments include Yescarta[®]▼ (the efficacy results were benchmarked against historical control datasets such as SCHOLAR-1, and post-authorisation safety study [PASS] will be set up), Kymriah[®]▼ (efficacy results were compared against three external data sets [SCHOLAR1, the CORAL extension study, and PIX301], and PASS and efficacy study will be implemented), Zalmoxis^{®*} (EBMT registry was used as a control group, and a PASS is ongoing), Strimvelis[®]▼ (PASS), or Soliris[®] (where upon the results of a global paroxysmal nocturnal haemoglobinuria registry a modification in the label was attained to extend the indication regardless of transfusion history).²

However, the use of real-world data also faces challenges such as data access, protection, sharing, and completion; inconsistent use of terminology; as well as potential biases and confounders that are not always captured, although possible solutions exist to all these problems.²

In summary, real-world data are playing an increasing role in healthcare decisions and can complement data from clinical trials to help inform patient care,² and regulatory agencies use real-world data for regulatory decisions and post-marketing safety monitoring. With appropriate consent, this RWE can provide additional insight into the safety and effectiveness of new treatments. Such information should be collected in classification and regression tree algorithms to demonstrate their longer-term safety and effectiveness. Real-world data can be used for clinical trial design development and observational studies, with electronic health records facilitating simultaneous data collection and safety monitoring without direct patient contact for large populations.^{5,7}

Real-World Experience with Phosphoinositide 3'-Kinase δ Inhibitor Therapy in Follicular Lymphoma and Chronic Lymphocytic Leukaemia

Doctor Manuela Hoehstetter and Professor Loïc Ysebaert

The presentation was started by Dr Hoehstetter by describing the efficacy of idelalisib, which is a first-in-class, selective, oral, phosphoinositide 3'-kinase δ (PI3K δ) inhibitor that acts by decreasing downstream signalling of the B-cell receptor and cytokine receptors (CXCR4, CXCR5, CXCL12), critical components of signalling pathways in B-cell malignancies.⁸ Idelalisib is approved in Europe for chronic lymphocytic leukaemia (CLL), in combination with rituximab or ofatumumab for adult patients with ≥ 1 prior therapy or as first-line therapy in the presence of *del(17p)/TP53* mutations in patients not eligible for any other therapies.⁹ Idelalisib is also approved as monotherapy for follicular lymphoma (FL) in adult patients refractory to ≥ 2 treatment lines.⁹ European Society for Medical Oncology (ESMO) guidelines currently place idelalisib plus rituximab as a treatment option for patients with relapsed CLL¹⁰ and idelalisib monotherapy for double-refractory FL.¹¹

*After the symposium, on 9 October 2019, the European Commission withdrew the marketing authorisation for Zalmoxis (nalotimagene carmaleucel) in the European Union (EU).³⁴

Study 116 was a randomised, controlled, Phase III trial of idelalisib plus rituximab versus rituximab alone (N=220) in patients with early relapsed CLL, medical comorbidities, and limited treatment options.¹² Patients enrolled in Study 116 could continue to receive idelalisib in the extension Study 117. In these trials, the median progression-free survival with idelalisib plus rituximab was 19.4 months versus 6.5 months for rituximab alone, whereas the median overall survival (OS) was 40.6 months for patients who received idelalisib plus rituximab and 34.7 months for patients who received rituximab.¹³ An analysis of patients stratified by *del(17p)/TP53* mutation status showed no impact on idelalisib plus rituximab clinical outcomes ($p=0.9012$).¹³ In the separate Study 101-09, idelalisib monotherapy also showed clinical benefit in patients with heavily pretreated FL, with an 11.0-month progression-free survival compared with 5.1 months with the prior regimen.¹⁴

Real-world data showed similar results compared with trial data. Retrospective data from the Polish Adult Leukemia Group in patients with relapsed/refractory CLL (N=34) showed similar OS results to Study 116/117 data,¹⁵ whereas retrospective data from the UK and Republic of Ireland showed similar clinical benefit to trial data both in patients with CLL¹⁶ and FL.¹⁷ A prospective real-world data study to assess the use of idelalisib in routine clinical practice was conducted in Germany. After a median observation time of 11.5 months in the CLL cohort (N=84), the median OS was not reached, whereas the 12-month survival rates for patients with and without *TP53* aberrations were 81.0% and 83.0%, respectively. Furthermore, the PASS study also showed that prophylactic treatment for *Pneumocystis jirovecii* pneumonia (PJP) improved survival, with 12-month OS rates of 84.0% versus 76.0% in patients with and without prophylaxis, respectively.¹⁸

The presentation was continued by Prof Ysebaert, who summarised safety data of idelalisib collected both in clinical trials and real-world studies. In clinical trials, treatment discontinuation due to adverse events occurred in 25–29% of patients.^{13,14} The most common adverse events occurring both in patients with FL and CLL were diarrhoea/colitis, increased liver transaminases, neutropenia, and infections.^{13,14} Pooled data from multiple idelalisib trials showed that prophylactic treatment for PJP infection is required.¹⁹ Currently, the European Medicines Agency (EMA) recommends

prophylaxis to be used in all patients throughout idelalisib treatment, and for a period of 2–6 months after discontinuation.⁹ Other common adverse events leading to discontinuation can be occasionally managed by re-challenge with a lower dose. Based on clinical trial data, 41% of patients with interruptions due to Grade 3 diarrhoea/colitis and 83% of patients with Grade 3 transaminase elevations were successfully re-challenged and continued treatment.¹³

Published real-world data generally support safety findings from clinical trials. Retrospective data from the UK and Republic of Ireland that included patients with a performance status ≥ 2 revealed the same pattern of adverse events as in the clinical trials, but at lower rates.^{17,18} Furthermore, a French retrospective, multicentre survey study of idelalisib patient management showed similar adverse event profiles in patients with FL (n=145) and CLL (n=384) compared with clinical trial data, but with larger discontinuation rates due to adverse events.²⁰ In this French retrospective study, multivariate analyses showed that good performance status and anti-PJP prophylaxis were associated with longer treatment duration.²⁰ Furthermore, patients who had at least one nurse follow-up tended to have a longer treatment duration both in FL and CLL, demonstrating the importance of nurse follow-up on patient persistence in voluntarily taking the treatment.

In summary, the efficacy and safety of idelalisib were established in clinical trials. Consistent with trial data, several years of RWE showed similar clinical outcomes with no new safety signals. Finally, anti-PJP prophylaxis should be administered to all patients receiving idelalisib, neutrophil count and CMV surveillance is recommended, and nurse consultations may help with increasing patient adherence to treatment.

Real-World Experience with Chimeric Antigen Receptor T cells in Aggressive Non-Hodgkin's Lymphoma

Professor Yi Lin

The SCHOLAR-1 retrospective study used pooled data from clinical trials and observational studies in relapsed/refractory DLBCL, and showed a median OS of 6.3 months, a 2-year OS of 20%, and a complete response (CR) rate of 7%,²¹ demonstrating a high unmet need in these patients. However, recent developments in CAR T-cell therapies have brought a new viable option for these patients.

The pivotal multinational ZUMA-1 trial enrolled patients with refractory DLBCL, primary mediastinal large B-cell lymphoma, high grade B-cell lymphoma, or transformed FL. Patients enrolled in the study underwent leukapheresis for manufacturing of CAR T cell and received lymphodepleting chemotherapy followed by treatment with axicabtagene ciloleucel (2×10^6 anti-CD19 CAR T cells/kg body weight). A total of 111 patients were enrolled and underwent leukapheresis. The manufacturing success rate of the treatment was 99%, and 91% of patients received treatment.²² After a median follow-up of 27.1 months, the overall response rate (ORR) and CR per independent central review committee among treated patients were 74% and 54%, respectively, and the median OS was not reached.²³ The most common adverse events included cytokine release syndrome (93%; 11% Grade ≥ 3) and neurological events (67%; 32% Grade ≥ 3).²³ The majority of these common adverse events were reversible and can be managed with established protocols and informed management.²⁴

Retrospective studies of real-world practice have reported that patients who did not meet the eligibility criteria from the pivotal ZUMA-1 trial have been treated with axicabtagene ciloleucel, including patients with an Eastern Cooperative Oncology Group (ECOG) performance status >1 , thrombocytopenia ($<50,000/\mu\text{L}$), reduced kidney function (serum creatinine >1.5 mg/dL), liver abnormalities (serum alanine transaminase/aspartate transaminase ≤ 2.5 ULN; total bilirubin ≤ 1.5 mg/dL), or a history of central nervous system lymphoma. In all patients evaluable after 30 days of treatment (n=112), the ORR and

CR per investigator assessment were 79% and 50%, respectively, whereas in patients evaluable after 100 days (n=39), the ongoing response and CR were 59% and 49%, respectively. Grade ≥ 3 cytokine release syndrome occurred in 7% and neurological events in 31% of evaluable patients (n=163). Despite the inclusion of patients with clinical characteristics outside of pivotal trial eligibility, the safety and efficacy results were similar compared with ZUMA-1 results.²⁵ Another USA retrospective cohort analysis showed that axicabtagene ciloleucel-treated patients (N=73) had a best ORR and CRR of 64% and 41%, respectively, at a median follow-up of 4 months, with Grade ≥ 3 cytokine release syndrome and neurological events observed in 17% and 38% of patients, respectively.²⁶ Finally, another retrospective analysis performed at a single USA centre showed similar response rates with the same adverse event profile for patients younger and older than age 65, indicating that an age >65 did not preclude treatment with axicabtagene ciloleucel.²⁷

Navigating the logistics of upfront insurance approval, pre-treatment evaluation, and leukapheresis can impact access to axicabtagene ciloleucel. In the USA, a single-centre study looking at the experience with 13 patients showed that the median time from patient referral to autologous anti-CD19 CAR T-cell infusion was 37 days (range: 31–80) days, which included a median 12 days for insurance approval, showing that opportunities still exist to decrease the pre-treatment timelines for these patients, which can also be variable across different health systems.²⁸ Furthermore, a survey conducted at 26 certified centres to prescribe CAR T-cell therapies showed that product logistics (including manufacturing time) influenced prescribing practices in 84% of centres, whereas the single most important reason informing treatment decisions was the adverse event profile (32% of centres).²⁹ This survey also showed a considerable heterogeneity in the use of the different toxicity grading systems and management.

In conclusion, anti-CD19 CAR T-cell therapy is an effective treatment option in patients with relapsed/refractory DLBCL. ZUMA-1 showed high rates of durable responses with axicabtagene ciloleucel, and early real-world studies supported the results of the trial and showed promising safety and efficacy results in a broader patient population.

Table 1: Interests of registry users.

Science and education	Quality control of clinical care	Safety surveillance
Data generation Courses Working parties Clinical trials International and national study groups	Benchmarking Donor registries National registries Accreditation	Clinicians Regulators Corporate sponsors

Harnessing the Power of Patient Registries

Mr Eoin McGrath

The EBMT is a Dutch-registered, non-profit organisation that includes 583 centre members located in 63 different countries, comprising a total 2,898 physicians, 826 nurses, and 645 data managers.³⁰ The EBMT was established in 1974 and currently contains data for over 666,000 haematopoietic stem cell transplants, with information on more than 40,000 haematopoietic stem cell transplants included in 2018 alone.³⁰

The interests of the registry users include science and education, quality control of clinical care, and safety surveillance, which is of importance to clinicians, regulators, and corporate sponsors (Table 1). The benefits of patient registries include case identification for prospective and retrospective studies, real-world assessment (therapy effectiveness), risk factor insights, improved patient management, healthcare services assessment, patient subgroup evaluation, and institutional benchmarking.³¹

As more and more patients are becoming survivors of cancer, long-term follow-up is needed, which is often not performed as part of clinical trials. As an example, several large cohort studies have shown an increase in secondary malignancies and cardiovascular disease following radiotherapy and chemotherapy, which were not captured as part of clinical trial monitoring.³² Furthermore, registries can also offer a ‘neutral ecosystem’ for monitoring the impact of novel drugs on treatment choices. Patient registries also come with several challenges, including quality management, data

analysis (that can introduce bias), data security including General Data Protection Regulation (GDPR) compliance, validity of results, long-term retention, and patient follow-up, as well as in the assessment of adverse events.³³

Registry data have been useful to provide control patients for EMA assessment of a single-arm pivotal trial. Zalmoxis* treatment was assessed in a pivotal single-arm Phase I/II trial; because no control arm was included, retrospective match-pair analyses comparing trial patients with patients receiving standard of care and included in the EBMT registry were essential for marketing approval of Zalmoxis*.³⁴ Furthermore, data from the pivotal single-arm ZUMA-1 trial of axicabtagene ciloleucel was also compared with historical controls based on pooled analyses in patients with refractory non-Hodgkin’s lymphoma included in the SCHOLAR-1 analysis, leading to marketing approval.³⁵

Registries can be well placed to collect data for cell therapies, with manufacturers, payers, regulators, and clinicians all benefiting from these data. In the case of CAR T cell therapy approval by EMA, post-authorisation systems and risk mitigation strategies were specific requirements.³⁵ A similar registry from the Center for International Blood and Marrow Transplant Research (CIBMTR) is being implemented in the USA.³⁶ Based on EMA requirements, data from CAR T patients could be centralised in one registry to permit one point of reporting and data access for EU clinicians, and for supporting specific studies.³⁶

In summary, the use of patient registries for CAR T-cell therapies may change the lifecycle paradigm for future advanced therapy medicinal

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products. These patient registries were built by clinicians for clinicians to allow centralised data collection that can inform clinical decisions and serve to enhance patient care. A CAR T-cell therapy registry is needed for collecting data and performing pharmaco-epidemiological studies. Collaboration between the registry and all stakeholders, including clinicians, regulators, payers, and sponsors, is the key to future success.

Using Real-World Experience in Future Drug Development

Professor John P. New

The current EU trial pathway can be slow and costly. Preclinical testing followed by clinical trials can take between 9 and 13 years, which is followed by regulatory and payer approval of the new therapy, which can take another 2–5 years, for an approximate total cost of medicine development of \$2–4 billion. Patients can only have access to the new therapy once the process is complete.³⁷ Timely access to a promising treatment is relevant for any serious disease, regardless of its time course. In case of certain diseases, such as hypercholesterolaemia or dyslipidaemia, a long development timespan might not have a high impact for patients because the evolution of the disease towards major cardiovascular events is slow. This is not the case for patients with diseases like cancer for which quick access to new therapies is essential due to the high disease mortality.³⁸

External influences are pushing for a transition towards new drug development pathways that can lead to improved and quicker medicines for patients. First of all, patients demand timely access with an emphasis on diseases with an unmet need; second, emerging scientific research has led to a fragmentation of treatment populations based on genotypic biomarkers and early disease interception; third, the high costs of new therapies has led to a rise in payer influence; and finally, all these factors have placed

pharmaceutical sponsors under pressure affecting the sustainability of drug development.³⁸

The adaptive pathways approach to the drug development lifecycle may provide patients with timely access to beneficial medicines, by identifying patients most likely to benefit from new therapies and to adequately determine the evolving information on the risk-benefit ratio.^{38,39}

Furthermore, the adaptive pathways approach can also involve a transition in the way the research and development roadmaps are set up. The traditional development strategy starts with a large population, aiming to obtain an approval licence as broad as possible, followed by later assessment of patient subgroups that can derive the highest benefit from treatment; the adaptive pathway strategy would have an initial goal of showing a positive benefit-risk ratio in subpopulations with a high unmet need, and further studies would follow to support broader coverage and other indications.³⁸

Axicabtagene ciloleucel and tisagenlecleucel were the first therapies to be supported through the EMA PRiority MEDicines (PRIME) scheme to receive marketing authorisation based on small, single-arm studies with small patient populations with a high unmet need. Key risk management steps were advocated as part of the authorisation, including the mandatory utilisation of a patient registry to monitor long-term clinical benefit and safety, and a post-authorisation safety surveillance programme will report data until 2038.^{35,40} The use of electronic medical record data to demonstrate longer-term safety and efficiency of new treatments will provide essential information in the adoptive licensing process.

In conclusion, the current EU trial pathway can be costly and slow, but regulators are looking into new ways to balance the needs of patients, needs for safety, and the needs of the corporate trial sponsors. Adaptive approaches to drug development using RWE to determine the safety profile will ultimately help patients with a high unmet need receive new treatments in a timely manner.

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CAR-T: From Bed to Bench and Back Again

This symposium took place on 18th June 2019 as part of the International Conference on Malignant Lymphoma (ICML) in Lugano, Switzerland

Chairperson:	Claire Roddie ¹
Speakers:	Claire Roddie, ¹ Christian Chabannon, ^{2,3} Chris Shen, ⁴ John Rossi, ⁵ Louis van de Wiel ⁴
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Meeting Summary

The Kite, a Gilead Company, symposium “CAR-T: From bed to bench and back again” took place during the 2019 meeting of the International Conference on Malignant Lymphoma (ICML) in June. It was introduced by Chairperson Dr Roddie, who defined the meeting objectives: to provide an understanding of the science and key translational findings behind chimeric antigenic receptor (CAR) T-Cell development and to discuss the manufacturing process for the CAR T-cell product and the challenges involved in scaling this process from the laboratory to a commercial setting. Mr Rossi presented on the autologous anti-CD19 CAR T-cell therapy development journey and highlighted how

collaborations drove this, with academia and the National Cancer Institute (NCI) being under a cooperative research and development agreement (CRADA), and discussed the pivotal ZUMA-1 clinical trial. Dr Roddie followed by describing CAR T-cell therapy development from patient selection to apheresis, highlighting the importance of patient selection and the need for consensus guidance to standardise apheresis between centres. Dr Shen and Mr van de Wiel discussed the manufacturing process for axicabtagene ciloleucel production and how its patient-focussed nature has led to a drive to reduce product turnaround times, leading to manufacturing network expansion in Europe. This talk was followed by a presentation by Prof Chabannon, who explored how product release testing is vital to ensure drug product quality, highlighting how the CAR T-cell manufacturing process differs from that of conventional drug products. He also noted the importance of release tests and quality control (QC) and discussed out-of-specification (OOS) products, and how these can be managed. Finally, Dr Roddie discussed pretreatment patient management and the infusion process, noting that lymphodepleting chemotherapy is critical to the CAR T-cell administration algorithm. She concluded by discussing the challenges in 'scaling out' CAR T-cell manufacture to meet patient needs, particularly good manufacturing practice (GMP) cell manufacturing and scaling out quality systems to ensure a standardised process for high-quality treatment in all qualified sites.

Cell Therapy Development Journey: From Bench to Bedside

Mister John Rossi

The initial CAR concept was developed as early as the 1980s.¹ Later, CAR research took place under an accelerated timeline. In 2010, the NCI published the first case report of successful anti-CD19 CAR T-cell therapy in a patient with non-Hodgkin's lymphoma, demonstrating for the first time antitumour efficacy and also an on-target off-tumour effect of prolonged B-cell depletion after therapy.² Following this initial study, Kite and NCI entered into a CRADA relating to an initial study in a cohort of 22 patients who were treated with an anti-CD19 CAR, preceded by low-dose chemotherapy.³⁻⁵ The overall response rate was 73%, with a complete response seen in 55% of patients. In an extended follow-up of the NCI trial, four of five patients with initial complete remission had long-term duration of remission ranging from 38 to 56 months.⁶ The U.S. Food and Drug Administration (FDA) granted the 'breakthrough' biologic license application of axicabtagene ciloleucel on 18th October 2017.

The pivotal ZUMA-1 study started in April 2015. This Phase II multicentre study included 101 patients in two cohorts: the first with refractory diffuse large B-cell lymphoma (DLBCL [cohort 1; n=77]) and the second with refractory primary mediastinal large B-cell lymphoma (PMBCL) or transformed follicular lymphoma (cohort 2; n=24). Key eligibility criteria were no response

to last chemotherapy or failed autologous stem cell transplant (relapse within 12 months post-autologous stem cell transplant), and prior therapy with anti-CD20 monoclonal antibody and anthracycline.⁷ Cells were successfully manufactured for 99% of the patients enrolled and 91% of those enrolled were dosed, demonstrating product reliability and therapeutic feasibility.^{8,9} After 1 year, 82% of the patients who received infusions showed an objective response and 54% of the patients showed a complete response. Grade 3 or higher cytokine release syndrome (CRS) and neurologic events occurred in 13% and 28% of the patients, respectively.⁹ A subsequent analysis showed durable responses with a median follow-up of 27.1 months, with 39% of patients still having an ongoing response. Median time to response was 1 month (interquartile range: 1-1). The median overall survival (OS) was not reached with a median follow-up of 27.1 months (95% confidence interval [CI]: 12.8, not estimable [NE]), and the OS rate was 51%; the stability of the Kaplan-Meier curve for OS probability at the 0.5 point demonstrates the effectiveness of CAR T-cell technology in this very ill patient population (Figure 1). At the 2-year data analysis, Grade 3 or higher CRS or neurological events were reported in 11% and 32% of patients, respectively. No new axicabtagene ciloleucel-related CRS or neurological events were reported in the extended analysis at 2 years. Grade 3 or higher infections occurred in 28% of patients. Grade 3 or higher neutropenia, thrombocytopenia, and anaemia adverse events occurred in 39%, 24%, and 46% of patients, respectively.

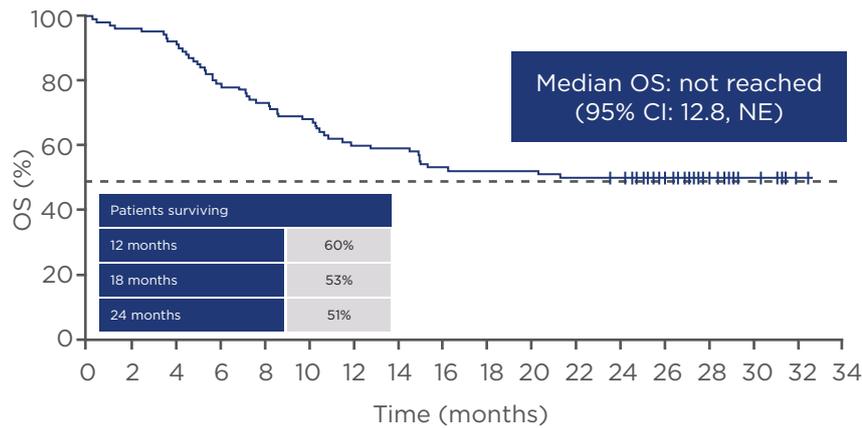


Figure 1: Overall survival over time with axicabtagene ciloleucel.

CI: confidence interval; NE: not estimable; OS: overall survival.

Adapted from Locke FL et al.⁸

The rates of the identified risks (CRS, neurologic events, infections, and cytopenias) were similar to those in the primary analysis.^{8,10}

The results showed that early expansion of anti-CD19 CAR T cells (measured by anti-CD19 CAR T-cell peak blood levels within the first 7-14 days) positively correlate with objective responses and neurological adverse reactions.^{9,10} Rapid expansion of CAR T cells from baseline was seen within 7-14 days after infusion. By 24 months, 11 (34%) of the 32 assessable patients maintained ongoing responses but no longer had detectable gene-marked CAR T cells.

On the other hand, 75% of the assessable patients with ongoing responses showed evidence of B-cell recovery, and initiation of B-cell recovery was noted in some patients as early as 9 months after infusion,⁸ suggesting that a patient would not need to have functional CAR T cells present to maintain a durable remission. In the USA, axicabtagene ciloleucel received approval from the FDA in October 2017.¹¹ Axicabtagene ciloleucel, marketed as Yescarta,[®] is a diverse polyfunctional product that is clinically effective across a broad range of CD4:CD8 ratios and comprises phenotypically naïve, central memory, effector, and effector memory T cells.^{5,9} Yescarta showed clinical efficacy across subsets with poor prognosis, including age, disease state (III/IV), high International Prognostic Index (IPI), bulky disease, cell-of-origin, and double-/triple-hit

lymphoma.^{8,9} European Union (EU) marketing authorisation was received in August 2018 for the treatment of adult patients with relapsed or refractory DLBCL and PMBCL after two or more lines of systemic therapy.¹⁰

In summary, ZUMA-1 has a median follow-up in lymphoma patients of 27.1 months^{8,9} and the OS was not reached. Durable responses were observed at the 2-year follow-up analysis. Two-year analysis of the multicentre ZUMA-1 trial shows that a substantial proportion of patients with refractory LBCL treated with axicabtagene ciloleucel can achieve durable responses with manageable long-term safety.⁸

From Patient to Commercial Production: Apheresis to Sample Shipping

Doctor Claire Roddie

Patient selection is a vital part of the CAR T-cell production process.¹² Patients eligible for treatment with axicabtagene ciloleucel are those with relapsed/refractory DLBCL or PMBCL post two or more lines of systemic therapy. In the pivotal ZUMA-1 trial, patients were required to have adequate organ function, creatinine clearance (Cockcroft-Gault) ≥ 60 mL/min, serum alanine/aspartate transaminase ≤ 2.5 upper limit of

normal, total bilirubin ≤ 1.5 mg/dL, ejection fraction $\geq 50\%$, no clinically significant ECG findings, no clinically significant pleural effusion, and baseline oxygen saturation $>92\%$ in room air.⁹ Further, it has been suggested that patients should be relatively fit and without serious comorbidities, willing to travel to a specialist centre for CAR T-cell therapy if needed, and have an appropriate home support network, including care support for the acute period (30 days) after CAR T-cell infusion when the risks of neurological adverse events are highest.^{10,13}

Lymphapheresis is a critical step in obtaining starting material for a high-quality CAR T-cell product; therapy should be halted in a timely fashion before apheresis to maximise chances of adequate CD3 yield. A target recommended minimum blood volume of roughly 10–20 L is processed to deliver a final volume of apheresis of approximately 100–500 mL, with a target of approximately $5\text{--}10 \times 10^9$ mononuclear cells.^{5,8,14}

Duration and time since last exposure to prior therapies that may impact lymphocyte count should be considered before apheresis (i.e., checkpoint inhibitors, ibrutinib, chemotherapy, and radiotherapy), and use of corticosteroids must also be considered because of their potential lymphotoxicity. Systemic chemotherapy, radiation therapy, and immunosuppressive therapy should be halted in advance of apheresis. At least 2 weeks or 5 half-lives, whichever is shorter, must have elapsed since any prior systemic therapy at the time the patient is planned for leukapheresis, except for systemic inhibitory/stimulatory immune checkpoint therapy.⁸ Corticosteroids should be avoided 7 days before apheresis. Other medicines with systemic inhibitory/stimulatory immune activities, such as checkpoint molecule therapy, should be stopped approximately 3 half-lives in advance. Intrathecal therapy should be eluded 1 week in advance.^{8,14} Patient blood composition can impact lymphocyte yield in the apheresis; high proportions of natural killer cells or myeloblasts, and low absolute lymphocyte count in peripheral blood, can compromise CD3 yield and lead to a low-target apheresis harvest; low CD3+ T-cell yield can lead to impaired CAR T-cell expansion *in vitro*.¹⁵ Although the percentage of T cells in the starting materials may vary widely, with the result being that some patients were essentially lymphopenic, the resulting T-cell products were highly enriched for CD3+ T cells in ZUMA-1.¹⁶

Shipping via cold chain is the final piece of the process; on the day of apheresis, Kite provides a NanoCool® container for shipping. Apheresis material is collected from hospital laboratories and transported to one of Kite's manufacturing locations for peripheral blood mononuclear processing.¹⁷ The KiteKonnnect® online portal provides a range of supporting features to hospitals and healthcare providers to help facilitate the treatment and manufacturing process, including patient enrolment, co-ordination calls, planning of apheresis, and product tracking.

The chain of identity and chain of custody (tracking of one patient, one apheresis material) are critical when treating patients with autologous cell therapy products, and excellent communication between the manufacturer and the treating centre is essential. In ZUMA-1, median turnaround time was 17 days; therefore, bridging therapy was not allowed to enable a better assessment of the efficacy results.⁹ However, bridging therapy may be needed between apheresis and CAR T-cell therapy infusion whilst patients are awaiting the processed cells, as has been demonstrated in more than half of patients in the real world in the USA.^{12,18} Patient management should be collaborative between the CAR T-cell treatment and referring centres; preferred therapies are those that will not make patients unwell or negatively impact their performance status. Consensus guidance around standardisation of apheresis processes may help to provide uniformity of CAR T-cell products across treating centres and manufacturers.

From Sample Receipt to CAR T-cell Product

Doctor Chris Shen and Mister Louis van de Wiel

The axicabtagene ciloleucel product flow is an aligned process between the manufacturer, the apheresis unit, and the treating hospital to return the product to the patient timely. Teamwork is vital and an efficient manufacturing process requires seamless integration and communication between multiple stakeholders, including physicians and Kite internal teams. The flow starts at the Kite manufacturing facility with generation of the chain of identity and then proceeds with shipping of the apheresis materials

kit to the relevant apheresis unit, where the chain of custody starts. After the cells are collected, they will be shipped to the manufacturing facility and inspected for quality and condition before manufacturing begins. QC testing is also a vital part of all the steps in the production process.¹⁶

In ZUMA-1 at the USA sites, the manufacturing success rate was 99% with a median turnaround time of 17-18 days.⁹ In Europe, the turnaround ranges from 26 to 29 days, mainly due to the transport time needed between Europe and the USA for cell processing.¹⁹ The USA real-world manufacturing success rate is 97%.¹² Kite is based in California, with research and development, and commercial and clinical manufacturing facilities in the Los Angeles area in the USA. Kite also has entered into joint ventures/license collaborations with companies in Japan (Daiichi Sankyo)²⁰ and China (Fosun Kite).²¹ Kite is committed to increasing patient access in Europe, and manufacturing network expansion is underway. A new facility in the Amsterdam area of the Netherlands will be operational by the beginning of 2020 and will supply the European market, with the capacity to produce approximately 4,000 patient treatments per year.²²

The CAR T-cell therapy process begins with apheresis, which can be highly variable depending on patient status and collection site. This step is followed by T-cell isolation and enrichment. As patient materials can have variable volume and cell concentrations, volume normalisation may be performed as the first step to 'standardise' starting material. The difference in manufacturing time between the USA and the EU is due to the current EU need for peripheral blood mononuclear cells to be cryopreserved and shipped to the USA. T cells are then activated to provide the primary signal; this is then followed by gene transduction of T cells (engineering with CAR). Anti-CD19 CAR genes are introduced into cells by retroviral transduction.¹⁶ CAR T-cell expansion then follows. A single dose of axicabtagene ciloleucel contains a target dose of 2×10^6 CAR-positive viable T cells per kg of body weight (range: 1×10^6 – 2×10^6 cells/kg; up to a maximum of 2×10^8 CAR-positive viable T cells for patients ≥ 100 kg) in approximately 68 mL dispersion in an infusion bag.¹⁰

T-cell activation, transduction, growth, and final formulation are all critical to an efficient manufacturing process. Expansion is then followed

by QC testing.^{5,16} Cell growth rates can be variable depending on the starting product (the growth process takes 4-7 days), but the manufacturing process is designed to accommodate this.

Kite has developed a robust and efficient approach to engineered cell therapy using the patient's own T cells, and a process enabling consistent, timely delivery of a high-quality product. The future will involve continuing process development with an emphasis on automation, product consistency, increased efficiency and capacity, and decreased manufacturing turnaround time.

Product Release Tests: Helping Ensure Patient Safety and Guaranteeing Drug Product Quality

Professor Christian Chabannon

There are essential differences between the CAR T-cell manufacturing process and the production of conventional drug products. Traditional manufacture of drugs begins with fully characterised and batched starting materials. It is large-scale and mostly automated, resulting in thousands of similar lots for release and distribution. CAR T-cell manufacturing, by contrast, is individualised to each patient (batch size of one), has an inherent variability of starting product, a complex manufacturing process (which is currently mostly manual), uses a specialised facility with highly trained personnel, and rarely encompasses OOS production with exceptional release.²³⁻²⁵ As CAR T-cell manufacturing begins with heterogeneous starting material, release tests and QC of all products are vital to ensure its safety and effectiveness.²⁶ After the CAR T-cell manufacturing process in the USA, the cell product is passed to QC for testing, which comes out 7 days after formulation, and the product is then cryopreserved and shipped back to the European site for batch release and treatment. Before release, a qualified person ensures that each manufactured product batch meets the specifications required by the European Commission.²⁵

During axicabtagene ciloleucel production, each CAR T-cell product undergoes a series of

batch-release tests for viability, purity, potency, microbiological safety, appearance, and identity.^{5,16} Lot release testing serves as the final confirmation of product quality before release for use; these specifications ensure a consistent final product.¹⁷ Potency assays are an essential component of release testing. Specifications are agreed upon with competent regulatory authorities during the marketing authorisation process; CAR T-cell potency assays should demonstrate CD19 antigen recognition, T-cell effector function and activation, and be representative of the product mechanism of action.

Increased manufacturing capacity within the new Kite European facility in 2020²² should result in removal of the need to ship to the USA and back and, in the best case scenario, align the EU manufacturing time in a similar fashion to that seen in the USA (17–18 days). There are challenges associated with autologous cell therapy manufacturing; it is a specialised process with inherent variability. In real-world experience in the USA, around 3% of the cases received a nonconformity product in the context of ZUMA-9.¹² These products are designated OOS.^{23,24} All medicinal products should be manufactured in compliance with GMP, according to the guidelines for advanced therapy medicinal products (ATMP)-specific GMP published in 2017.²³ The Committee of Advanced Therapies (CAT) has recently issued a ‘questions and answers’ document on the use of OOS batches of authorised ATMP.²⁴ OOS products can still exceptionally be administered to avoid an immediate, significant hazard to the patient. This release is dependent on physician request, after which the manufacturer follows a set procedure based on ATMP-specific guidelines.²⁵ If the OOS product is not appropriate to be released exceptionally, then the manufacturing process can be restarted, sometimes even with a new apheresis procedure which may require additional management of patient disease during this extra period.

The individualised nature of CAR T-cell therapy introduces inherent variability at the start of the manufacturing process.²⁷ Apheresis variability may be among the causes of product variability.¹⁵ QC from the beginning to the end of the manufacturing processes of all CAR T-cell therapies is essential to ensure the safety and effectiveness of these products.²⁶

In conclusion, the axicabtagene ciloleucel manufacturing process is robust, with success rates of 99% in ZUMA-1⁸ and 97% in the real-world setting in the USA.¹² Although the majority of manufacturing events are successful, manufacturers should continue to improve their processes to minimise OOS production, and implement procedures for risk assessment and communication to the sites of the eventual OOS cases.²⁴

Scaling CAR T-Cell Therapy for a Good Manufacturing Practice Standard Product

Doctor Claire Roddie

The CAR T-cell infusion process starts with patient admission for lymphodepleting chemotherapy. A conditioning regimen of fludarabine (30 mg/m²) and cyclophosphamide (500 mg/m²) is used for standard lymphodepletion (over 3 days from Day 5 before CAR T-cell infusion; three doses of fludarabine and three doses of cyclophosphamide). This step in patient conditioning is essential for depleting endogenous lymphocytes and elevating homeostatic cytokines (i.e., IL-15 and IL-7).⁴ The blood bank and the patient should be informed of the requirement for irradiated blood products, and the treating physician should ensure that at least 2 days elapse between the last dose of lymphodepleting chemotherapy and CAR T-cell administration (to ensure the clearance of fludarabine from the system). All patients should be made aware of the risks of neutropenia and possibly sepsis with this conditioning regimen.¹⁰

At the time of infusion, hospitals should ensure the existence of checklists to cover all the processes. Patient identity must be checked before removing the bag from the cassette. Then, the bag must be thawed at 37 °C either in a water bath or using dry methods for 3–5 minutes. No further manipulation of the product (e.g., washing, resuspension, or radiation) is permitted. As required with other infusions of lymphocytes or haemopoietic progenitors, a leukodepleting filter must not be used.

During the postinfusion period, patients must be closely monitored for 4 hours for infusion reactions.

	Staff	<ul style="list-style-type: none"> • Training records
	Control of components	<ul style="list-style-type: none"> • SOP for handling and control of components • Acceptance criteria • System for traceability of all materials used in manufacturing
	Facility/equipment	<ul style="list-style-type: none"> • Clean room environment • Appropriate air handling, temperature control, and lighting • All equipment certified and maintained
	Batch manufacture records	<ul style="list-style-type: none"> • Complete written record of equipment (including maintenance and calibration) • Consumables/reagents • Manufacturing and testing (including microbiological sterility) • Deviations/procedural alterations/investigations/complaints • Quality control/qualified person reports • Product distribution
	Assays for release/stability	<ul style="list-style-type: none"> • Confirm the identity, strength, and purity of the product • Assays of stability • Assays of potency
	Labels and distribution	<ul style="list-style-type: none"> • SOP for packaging to prevent contamination and damage • SOP for labelling to ensure data integrity/allocation to correct patient • SOP for distribution and traceability

Figure 2: Scaling-out quality systems required of a current good manufacturing practice facility.

SOP: standard operating procedures.

Adapted from Roddie C et. al.¹⁵

Tocilizumab must be immediately available in case it is needed for management of CRS, which occurs within 1-12 days. Thus, patients must be monitored in a treatment facility for 10 days after infusion. The summary of product characteristics and the additional risk minimisation materials approved by competent national authorities provides guidelines for management of CRS and neurological toxicity.¹⁰

Why do we Need to Scale Out for a Good Manufacturing Practice Standard Product?

The CAR T-cell journey includes many steps such as apheresis, T-cell enrichment, gene transduction, T-cell expansion, formulation, and testing. Current commercialised CAR T-cell products are individualised to each patient and require a batch per patient, and therefore have a very different manufacturing process than that used in conventional drug manufacturing. 'Scaling out' of current CAR T-cell manufacture requires increased labour, materials, equipment, and space.²⁸ Challenges in scaling out include ensuring that CAR T cells are manufactured according to GMP requirements, a system by which medicinal products are produced safely

according to standardised methods under tightly controlled, reproducible, and auditable conditions. Fundamental quality systems required in a GMP facility include items described in [Figure 2](#).¹⁵

In the future, some of the current logistical challenges may be improved at different points of the process: eliminating the cool chain manufacturing requirement, harmonising national cell transport license variations, enhancing and simplifying digital tracking of individual identity of patient cells, implementing standardised global release procedures, and potentially identifying and removing unnecessary tests.

Cell therapy manufacturing is proceeding to overcome these challenges, with manufacturing capacity being scaled up, increased use of automation, highly trained staff, samples being tested and quality assured, site qualification, improved transport and logistics, and, importantly, maintenance of the chain of custody. Kite is currently deploying a standardised process of qualification of all the treatment sites, including steps such as audit, training in the additional risk minimisation materials, and a dry-run exercise to ensure high-quality treatment.¹⁷

Currently, autologous CAR manufacturing involves scaling out to produce more therapies, with replication of the same scale of equipment and processes performed in parallel. In the future, scale-up as per conventional treatments may involve an increase in batch size with 'batched' products from a single allogeneic source (a healthy source where multiple batches can be made from that single donor).¹⁶ Alternatives to viral transduction exist, which may remove one huge hurdle to CAR T-cell manufacture: the production of GMP-ready viral vectors to manufacture these cells. Thus, allogeneic production could remove

a lot of the complexity involved in the current autologous manufacture system.

In conclusion, the scaling out of autologous CAR manufacture to meet the needs of many patients in DLBCL has been shown to be feasible. The current manufacturing process is continuously undergoing improvements. Further automation is being investigated, which may give more benefit to overcome some of the challenges and shorten the turnaround time. Scale-out has already been successfully achieved with axicabtagene ciloleuce in more than 1,000 patients in clinical trials and in real-world treatment.^{10,12,18}

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